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THE CASTAWAY AT SEA

Nothing in tradition or in general experience can be said to hold that water of the open ocean is potable. Old sailors knew that small amounts were merely laxative but they also knew that large quantities were nauseating or emetic. And uncounted shipwreck survivors have discovered that man's thirst will not be quenched by sea water. Indeed, Aretaeus crystallized an ancient truth when he wrote, ". . . . thirst is engendered by saltish things."

Questions concerning water, food, and survival of the castaway at sea have always been poignant. Recently they have become pressing as a result of Alain Bombard's contending views ("Voyage of the Heretique," *Simon & Schuster, New York (1954)*). An advocate of the heretical idea that castaways short of fresh water should drink from the ocean, Bombard elected to test his convictions by crossing the Atlantic alone in a dinghy. After a preliminary voyage across the Mediterranean, he left the Canary Islands, drifting for sixty-five days before arriving at Barbados. In this period he subsisted on sea water, fish catches, fish juice and rain water. Articulately equating his success to proof of his theories, he attested, "I had conquered the menace of thirst at sea."

The possible consequence of this radical view for policies on proper advice for castaways has caused conservative physiologists to re-examine their stock of doctrine on this subject. It still appears as though there are good reasons to caution against drinking sea water, although they are hardly so compelling as those mathematicians give to deal with the perennial crops of circle-squarers and angle-trisectors who do not perceive the fundamental impossibility of their missions. It should be said at once that the physiologists' verdict on the related matter of diadermic drinking, *i.e.*, the supposed extraction of fresh water by the skin in contact with sea water, stands unshaken. This tenet could be dismissed out of hand as a crude fallacy, but it is also experimentally and observationally invalid. Likewise, the sea water enema, which enjoyed some attention from dipsologists during the two World Wars, is known to those who have tried it not as a source of free water but only as a regrettable experience.

In concentrating urinary solutes the human kidneys expend power of the order of $\frac{1}{30}$ watt. If wholly applied to the extraction of fresh water from sea water, this could provide 1200 ml. of fresh water per day. But the kidney has other, ineluctable obligations precluding this possibility, one of which is the allocation of much of its osmotic effort to the concentration of urinary nitrogen. The limited ability of the kidneys to regulate a diet of sea water may be deduced directly:

"Open ocean" water contains about 3.5 per cent of salts. Man concentrates his urine maximally to about 2 per cent of salt as sodium chloride. Ignoring minor defects in these premises, which do not alter essentially our conclusion, we infer that a man drinking 100 ml. of sea water might have to excrete 175 ml. of urine to remove the 3.5 g. salt load, suffering thereby a net loss of 75 ml. of water. If he did not lose all of the ingested salt he might (1) sustain a salt excess which would steadily augment for the worse with continued sea water drinking, and (2) suffer a higher than normal concentration of salts in his body fluids which, as is known, tends to provoke thirst.

A biologic objection to sea water drinking has been derived from consideration of the aquatic marine mammals. Seals and whales,

for example, avoid the ingestion of more than small quantities of sea water. If they don't see fit to drink it, the argument goes simply, man had better not. This introduces an interesting problem.

Twenty years ago, L. Irving, K. C. Fisher, and F. C. McIntosh (*J. Cell. Comp. Physiol.* **6,** *387 (1935)*) performed calculations which showed nicely that the seal could get all of its required water from its diet. "Herring" was the object food for the demonstration and seals got along on it exclusively in their experiments. "It is a relief to see," these workers concluded, "that the problem of physiological adaptation in the matter of the metabolism of water is provided for by the constant temperature of the environment, and that there is no evidence that so fixed a character as the mammalian kidney is modified in the adjustment of mammals to a marine habitat."

A. Krogh ("Osmotic Regulation in Aquatic Animals," *Cambridge University Press, Cambridge (1939)*) accepted the basic argument of these investigators along with most of their figures, and presented a parallel case favorable to the whale. There might appear to be less margin to spare in finding the necessary free water for the *Mystacoceti*, or whalebone whales, which obtain their water from plankton and other invertebrates than for the *Odontoceti*, or toothed whales, which feed largely on vertebrates, since the body fluids of invertebrates generally are isotonic with sea water whereas those of vertebrates are hypotonic. Actually, seals also may eat crustacea and molluscs; nevertheless, by Krogh's analysis all squeak through.

The heuristic weakness in calculations of this sort is that they merely rationalize a "safe" view. We already know the seal gets its water from food. Do the calculations really describe the mechanism of the seal's water economy? In point of fact it was but a happy circumstance that, using old nutritional tables of composition for the "herring," Irving *et al.* were able to support the idea that a "typical" mammalian kidney (of a carnivore) could manage its total excretory requirements with the preformed and oxidative water of herring. Of course, the fact that the seal need not expend water for temperature regulation helps.

It is easy to show by reference to modern nutritional tables ("Composition of Foods—Raw, Processed, Prepared," by B. K. Watt and A. L. Merrill, *U. S. Dept. of Agriculture, Agriculture Handbook No. 8, Washington (1950)*) that quite different results follow when calculations are based on "Atlantic herring," bluefish, halibut, Atlantic mackerel, or Pacific salmon. Theoretically, seals could *not* obtain sufficient water from these fish, while they could so obtain it from "Pacific herring," cod, flounder, haddock and clams. If we consider a diet of warm-blooded vertebrates such as may be taken by the killer whale or the leopard seal, judging its composition from that of raw beef, rib lamb chops, or hamburger, the case for free water becomes bleak indeed.

The seal can concentrate total urinary solutes to an osmolality of 2.4, about like the dog, and the latter can, to be sure, live well for long periods on raw fillet of haddock with no extra water. Both of these animals happen to be carnivores, but that fact is not crucial. Rat kidneys are somewhat better concentrators (to an osmolality of 2.6), and those of the cat may be still better (to an osmolality of 2.8). The renal emunctory of the kangaroo rat is unique, providing concentrations as high as 5.6 osmols per kilogram of water. This animal, which can concentrate its urine to the extent of 5.3 per cent of sodium chloride (total electrolyte perhaps to the equivalent of 8.8 per cent of sodium chloride), ordinarily lives its entire adult life without free liquid. It is quite able to drink sea water (1.0 osmolal) with benefit. Man, however, is one of the poorest urinary concentrators known among mammals, having less than two-thirds the total concentrating power of the seal. Superficially, man's urinary maximum of up to 1.5 osmols per

kilogram, exceeding by half the concentration of sea water, might appear to suggest his ability to excrete the salts of sea water satisfactorily, but much of the osmotic pressure of the urine derives from urea. The contribution of salt to the maximum urinary concentration is less than 0.6 osmols per kilogram. Thus, saltwise, man's urine is less than 60 per cent as briny as the sea, and this is the limiting factor.

Whether the seal can or cannot actually subsist indefinitely on pure diets of the fish categorized above is not known. No one has investigated the coefficient of digestibility of protein in these animals. Were it appreciably less than in man (the assumption of its equality being implicit in the calculations of Irving *et al.* and of Krogh), a new aspect to the water economy of these animals would be discerned. With greater rejection of protein in the feces, the effect would be as though the protein content of the diet were less, the urinary obligation of water for nitrogen excretion would be less, and more preformed water could be gained from the greater ingestion of fish which would be necessitated by its effectively lower caloric value. There would be less dependence on finely drawn but dubious assumptions in calculations of the water metabolism, and the seal could perhaps utilize the "theoretically" proscribed fish. On the other hand, the calculations may be valid, but remain to be put to the test.

In assessing the issue of whether sea water could or should be drunk by man, one may inadvertently suppose that advocacy of sea water drinking has implied its exclusive use. No one—not even Bombard, actually—seriously recommends such a course. (We omit from present consideration quite another idea concerning the virtues of sea water drinking, namely, W. S. S. Ladell's view (*Brit. Med. J.* **2,** *359* (*1954*)) that, from the standpoint of physical fitness rather than over-all survival time, there may be merit in drinking small amounts of that fluid.) Avoiding this pitfall, one addresses the question, can sea water be admixed with fresh water supplies to extend and profitably augment those rations? The value of fish juice is a related question.

G. R. Hervey and R. A. McCance (*Proc. Roy. Soc.* (*London*) *B* **139,** *527* (*1952*)) categorically advise that sea water should never be drunk by castaways short of fresh water on the ground that it is hypertonic to body fluids and appears to worsen hypersalemia at a time when this state is already in the making. Yet some observations suggest that certain admixtures of sea and fresh water serve better to quench thirst, better to maintain body water content, and better to augment the total water ration beneficially. The U. S. Navy admonishes the would-be survivor with these words on his sea water desalting kit, "Never Drink Sea Water!" Yet, by design, the potable water prepared with the standard silver zeolite briquets is practically a 1:9 admixture, or 10 per cent sea water; only 90 per cent of the osmotic activity is extracted in the desalting process. Researches during World War II suggested that not only was urinary water excretion less when some salt was ingested, but the salt hunger of starving men taking only fresh water was mollified.

It is generally agreed that eating raw fish or even attempting to chew the water out of the flesh (which results in simultaneous swallowing of protein) should be contraindicated for the man with limited fresh water supplies. However, the nature of the deleterious effects has not been completely assessed. It may be important to inquire whether under certain conditions a man does better to utilize the flesh of fish rather than his own flesh. In any case, a man is not a seal and cannot concentrate his urinary nitrogen sufficiently to enable him to gain free water from the fish. But if juice can be obtained by pressing the flesh of fish in a twisted rag or a fruit press, or by allowing it to collect in a hole cut in the sides of large specimens, should it not be used? It is almost certainly potable, per se, except where press-

ing does not sharply separate juice from pulp. Tribespeople of the desert of eastern Egypt are said habitually to drink waters containing up to the equivalent of 0.86 per cent sodium chloride, not too different from the body fluids of teleost fishes. If fish juice resembles osmotically a sea:fresh water mixture of 1:2 or 1:3, does this not suggest such mixtures would also be advantageous?

There is an important difference between fish juice and admixtures which, however obvious, cannot be overemphasized. When one makes a sea water admixture he adds only sea water to his total ration; when one obtains fish juice from a catch, he is in effect adding both sea and fresh water to his total ration since fish juice, abstractly speaking, is already a mixture of two virtual volumes of fluid, one osmotically equivalent to sea water and another of pure water. These make for vastly different physiologic situations.

The sea-fresh water admixture question must be considered separately. Suppose a man has 10 quarts of fresh water. Is he better or worse off to add 3 quarts of sea water (3.5 per cent salts, osmotically equivalent to 3.2 per cent sodium chloride) to give himself 13 quarts of a solution equivalent to 0.74 per cent sodium chloride? There is not space here for theoretic details but, in the writer's opinion, this matter has not been satisfactorily resolved. The psychologic argument that, under stress, the temptation to add more than allowable quantities of sea water would be insurmountable does not cut to the heart of the question, and the argument itself is debatable.

Experiments suggest that certain mixtures, such as those containing more than 30 per cent sea water, are deleterious to man. But good evidence is wanting for more dilute mixtures. A difficulty with mixtures which might be suitable in theory is that, if they are extremely dilute, the gain to be anticipated by admixing practically vanishes. Another important point, which has been made by Ladell (*loc. cit.*) and others, is that the important factors of ambient temperature and insolation (not to mention exercise), which heavily influence extrarenal water loss, affect the tolerable ratio of sea to fresh water. Were sea water indeed useful, then, in general, as heat gain from the environment or from metabolism is higher, relatively less sea water should be added to a given fresh water ration, despite the increased loss of salt in sweat. Not a paradox, this follows from the hypotonic nature of sweat, among other considerations.

It is surprising that the question of incorporating glucose in canned emergency water supplies has apparently not received much attention. The beneficial effect of glucose in minimizing urinary water loss through protein sparing and antiketosis, and the remarkable spiritual benefit it provides in raising the morale of the otherwise starving man is well known (J. L. Gamble, *Harvey Lectures* **42,** *247 (1946/47)*). The volumetric displacement of water when glucose is dissolved is almost equal to the water it yields on oxidation. Thus, within limits, this food does not compete spacewise with water stores. Whether there are unforeseen objections to sugar-water stores as life boat rations remains to be determined. Possibly some other carbohydrate, with or without flavoring, or certain soft drinks would actually prove more valuable than water alone.

The variety of fluids which the castaway will drink in the extremity of his thirst is endless: it most commonly embraces sea water, urine, and blood for their being most available. None of these perversions are of ultimate value. As has been broadly observed, there is no substitute for water.

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CELIAC DISEASE AND GLIADIN SENSITIVITY

Celiac disease is a chronic disturbance of nutrition, occurring in children under the age of 5 years, manifested by production of voluminous, fatty, foul-smelling feces, progressive emaciation of the trunk and extremities, marked abdominal distention, anorexia and irritability. Although bile acids and pancreatic enzymes are present in normal amounts in their duodenal juice, these children poorly absorb fat and carbohydrate from the intestine, as can be demonstrated by low blood sugar curves in oral glucose tolerance tests, and low rises in oral vitamin A tolerance tests. Malabsorption of calorigenic nutrients, vitamins and minerals leads to secondary deficiency diseases, and to marked retardation of growth and physical development. If untreated, the disease tends to run a protracted intermittent course over a period of several years.

Since 1950, significant contributions to the understanding of the pathogenesis of this disease have been made by workers in the Netherlands, whose earlier studies have already been discussed (*see Nutrition Reviews* **11,** *199* (*1953*)). Stimulated by the incidental observation that children with celiac disease appeared to react particularly unfavorably to the presence of wheat (or rye) in the diet, J. H. van de Kamer and H. A. Weijers (*loc. cit.*) showed that wheat flour exerts a harmful influence on celiac patients, causing anorexia, vomiting, apathy, diarrhea and steatorrhea. Later, they were able to prove that the protein fraction of the wheat, in particular gliadin, was the responsible factor. They therefore concluded that the cause of the patient's troubles, when eating wheat, must be sought in the metabolism of the wheat protein, steatorrhea being considered a secondary phenomenon.

These same workers have now reported studies which shed additional light on the cause of the harmful effect of wheat gliadin (J. H. van de Kamer and H. A. Weijers, *Acta paediat.* **44,** *465* (*1955*)), and provide a clinically applicable rapid test for wheat sensitivity (*Ibid.* **44,** *536* (*1955*)).

Because gliadin has an unusual amino acid composition, characterized by small proportions of lysine, methionine, threonine and other amino acids, and an unusually large proportion of glutamine (43 per cent), investigative efforts were directed toward determining the possible significance of the high proportion of glutamine, the monoamide of glutamic acid, in gliadin. Two 6 year old patients with celiac disease, in good clinical condition on a wheat-free diet, were given glutamine by mouth for six weeks, in doses increasing from 2 to 4 g. daily (equivalent to the glutamine content of 125 to 250 g. of wheat flour). No clinical or chemical reactions occurred, and these workers concluded that glutamine in the form of a free amino acid was not responsible for the manifestations of celiac disease.

The effects of protein-bound glutamine were next studied. Gliadin was chemically treated in such a way that it remained intact as a protein, while the glutamine was decomposed by a process of deamidation. The method used consisted of hydrolyzing gliadin by boiling it with 1 normal hydrochloric acid for forty-five minutes, causing 90 per cent of the glutamine to dissociate into glutamic acid and ammonia. The peptide bonds being affected to only a slight degree, the protein nature of the gliadin is thus preserved. This deamidated gliadin was given during three separate periods to 2 patients with celiac disease who had previously exhibited typical celiac reactions to a wheat diet. After deamidated gliadin had been substituted for gliadin in an equivalent amount, one patient completely recovered during both trial periods reported. The second patient, whose celiac disease was more severe, showed only transitory improvement. Because they suspected that the

residual 10 per cent glutamine in the gliadin might be intolerable to this patient, van de Kamer and Weijers prepared another hydrolyzed product, boiled for two hours instead of forty-five minutes in 1 normal hydrochloric acid, which contained only 3 per cent unchanged glutamine. When this product was given, the second patient's improvement was rapid, with sharply decreased steatorrhea, return of appetite, and disappearance of abdominal pain, diarrhea and pallor.

Based on these observations, a working hypothesis is proposed by van de Kamer and Weijers, which states that glutamine, bound in gliadin, but not as a free amino acid, is responsible for the nutritional aberrations of celiac disease. They have supported this hypothesis by determining the ratio of amide-nitrogen to nonamide-nitrogen in several foods. To establish these ratios the ammonia liberated by boiling for four hours with 1 normal hydrochloric acid was related to the total ammonia set free by a normal Kjeldahl treatment, according to the formula,

$$\frac{\text{amide nitrogen}}{\text{total nitrogen} - \text{amide nitrogen}} \times 100.$$

Foods which are well tolerated by patients with celiac disease (beefsteak, potato, whole egg, soybean oil meal, green peas, cod) all have ratios of 11 or less. In contrast, foods which disturb children with celiac disease (barley, rye, glutenin, wheat flour, gluten and gliadin) all have ratios of more than 11, the highest being that of gliadin, 30. Thus, the offensive foods are those with a high content of bound glutamine.

In their second recent paper (*Acta paediat.* **44,** *536 (1955)*), H. A. Weijers and J. H. van de Kamer describe their gliadin tolerance test for use in determining sensitivity to wheat. The fasting patient is given 350 mg. of gliadin per kilogram of body weight by mouth, in 15 to 20 ml. of buttermilk. The glutamine content of the blood is determined according to the method of B. A. Prescott and H. Waelsch (*J. Biol. Chem.* **164,** *331 (1946)*) on a micro scale in 0.3 ml. of plasma at zero, one, two, three, four and five hours after the test dose has been ingested.

Results of this test are reported for 13 normal children. None of the 13 showed an increase in blood glutamine concentration of more than 40 per cent above fasting level. Tests were also made on 29 patients with celiac disease, 15 of whom had been receiving wheat in their diets, and 14 of whom had been on wheat-free diets. The 15 patients who had been receiving wheat showed maximum increases in blood glutamine concentration ranging from 55 to 193 per cent, the mean increase being 104 per cent. The 14 patients on wheat-free diets showed maximum rises in blood glutamine concentration ranging from 22 to 121 per cent, the mean increase being 91 per cent.

The authors believe that, when their gliadin tolerance test is used, an increase of more than 50 per cent in the concentration of glutamine in the blood is indicative of sensitivity to wheat. They have found the test of particular value in outpatient clinical work, because it is more objective, and more rapidly performed, than the previously employed steatorrheic response to ingestion of wheat or gliadin.

THIAMINASE DISEASE

The occurrence of an active thiaminase in certain fish and shellfish is well known (*Nutrition Reviews* **10,** *134, 301 (1952)*). The production of a thiaminase by bacteria of the intestinal tract as an etiologic factor in the development of beriberi has received considerable study by a group of Japanese workers. Since this work has been reported largely in the Japanese literature, it has not received much attention in this country.

T. Shibata and S. Chang (*Proc. Vitamin B Res. Comm. (Japan)* **21,** *2 (1948)*; Chang, *Vitamins (Japan)* **2,** *174 (1950)*) observed that when a thiamine solution was placed in the sigmoidal colon of a patient who had habitual constipation combined with beriberi, the thiamine soon disappeared. This finding led to studies of the thiaminase of feces, which in turn led to the isolation of a new bacterium which was designated "*Bacillus thiaminolyticus* Matsukawa et Misawa" (BMM) (D. Matsukawa and H. Misawa, *Proc. Vitamin B Res. Comm.* **31,** *16 (1949)*; Y. Kuno, *Proc. Japan Acad. (Tokyo)* **27,** *362 (1951)*; **28,** *235 (1952)*). M. Fujimiya, Chang, and Matsukawa (*Proc. Vitamin B Res. Comm.* **38,** *22 (1950)*) reported that thiaminase was found in the feces of about 3 per cent of subjects studied. Its occurrence was more frequent in patients suffering from beriberi and gastrointestinal disease, and 15 per cent of 127 beriberi patients showed what these workers term thiaminase disease.

Some studies upon the distribution of thiaminase in the gastrointestinal tract, as well as its effect upon orally administered thiamine, are reported by D. Matsukawa *et al.* (*J. Vitaminology (Japan)* **1,** *43 (1954)*). The test for thiaminase involves determination of the amount of thiamine destroyed upon incubation of the material under standard conditions for two hours in the presence of 1 microgram of thiamine. In 2 patients who died of other causes but who were known to have thiaminase disease, samples of the intestinal contents were obtained at autopsy from different parts of the gastrointestinal tract. Considerable thiaminase activity was found from below the middle to the lower ilium in both these subjects.

Ten mg. of thiamine were administered daily to 5 patients with thiaminase disease and to 5 without the disease, and the fecal and urinary excretions of the vitamin were determined. Whereas from 60 to 80 per cent of the dose was recovered in the feces of the normal subjects and a total of approximately 85 per cent of the dose was found in the urine and feces combined, only 18 per cent was recovered from patients with thiaminase disease. Approximately 10 per cent was in the urine and 8 per cent in the feces. Other studies indicated that the ammonia and amino nitrogen excretions in the feces of the patients with thiaminase disease were much higher than in normal subjects.

In some subjects thiaminase could not be detected in the feces by the usual method, but the thiaminase-producing bacillus (BMM) could be cultured from the feces and the culture was then shown to contain thiaminase. These subjects are carriers, but for some reason do not develop appreciable numbers of the organism. The authors attempted to infect a series of 29 normal subjects with the organism by feeding a 4 mg. mass of BMM for several days. In only 2 of these individuals could thiaminase be detected in the feces and this disappeared spontaneously after administration of the bacillus was discontinued. D. Matsukawa, S. Chang, M. Fujimiya, and H. Misawa (*J. Vitaminology (Japan)* **1,** *No. 2, 53 (1955)*) did, however, produce thiamine deficiency in young hens by the administration of large amounts of BMM every third day for a forty-day period. Susceptibility to the BMM organism thus depends upon unknown factors, presumably the characteristic of the gastrointestinal secretions or the prevailing intestinal flora. These workers quote previous results to the effect that fecal thiaminase could be made to disappear by the administration of sulfanilamides or by the administration of lactic acid bacteria preparations. However, these treatments were only temporary, and thiaminase reappeared after therapy was discontinued. They also refer to studies of K. Hamada (*Vitamins (Japan)* **6,** *951 (1953)*; **7,** *65 (1954)*), who found that the presence of BMM may be related to the secretion of bile acids and that putrefactive bacteria are far more abundant in the intestinal tract of BMM carriers than in normal subjects.

A second thiamine-decomposing organism called "*Bacillus aneurinolyticus* Kimura et Aoyama" (or BKA) was reported by R. Kimura and S. Aoyama (*Vitamins (Japan)* **4,** *366, 461 (1951)*). A Fujita, Y. Nose, and K. Kuratani (*J. Vitaminology (Japan)* **1,** *1 (1954)*) state that the thiaminase formed by this organism has properties considerably different from those formed by the BMM organism or from those found in fish or plant sources.

Although the destruction of nutrients in the gastrointestinal tract has frequently been mentioned, it has seldom been given serious consideration as an important etiologic factor in the development of deficiency diseases. The work of Matsukawa and Chang and their co-workers is perhaps the first to implicate specific organisms in the development of a specific nutritional deficiency. It is clearly of importance to evaluate these findings more widely with respect to the occurrence of beriberi and to investigate the possibility that similar situations may occur with other nutrients. The complications that arise with respect to nutritional requirements and the epidemiology of nutritional disease are obvious.

DIURNAL VARIATIONS OF SERUM IRON

The effects of any procedure upon serum constituents need to be assessed against a stable base line. It is well appreciated that the functions of many organs undergo diurnal variations, but the marked changes in serum constituents have not always been so well recognized.

Recently, plasma iron turnover has been studied by a number of investigators using labeled iron (Fe^{59}). T. H. Bothwell and B. Mallett (*Clin. Sci.* **14,** *235 (1955)*) examined the effects of diurnal variation of serum iron on iron turnover rates. They studied 20 subjects, each on two separate occasions, in the morning and early evening. It was found that the diurnal variation could significantly change the iron turnover. In about one half of the cases, the turnover rate remained fairly constant because the change in plasma level was compensated by the rate of clearance of iron from the blood. However, when plasma iron clearance was rapid, as in the anemia of infection or uremia, the iron turnover reflected the influence of the serum iron concentration. The rates could vary between morning and evening as much as 50 per cent.

It was apparent from this study that the variability of individual responses makes it difficult to calculate iron turnover from observations of clearances of single tracer doses of iron.

Besides pointing up the effects of diurnal variations of iron on specific measurements, the problem of the diurnal variation of iron is again brought into focus. K. Høyer (*Acta med. Scandinav.* **119,** *562 (1944)*) examined the effects of the diurnal rhythm of serum iron in 41 subjects. He consistently noted the highest levels at 8 a.m. and the lowest between 8 and 11 p.m. There were no significant changes in levels of hemoglobin or serum proteins during these times. He then studied a group of nurses working at night. The highest serum iron level was seen in the evening after waking; the lowest in the forenoon, two hours after going to sleep. The magnitude of the variation was considerably less in these nurses than in the control group, suggesting that reversed waking-sleeping habits are not the only factors.

It was then of interest to explore the effects of changes in concentrations of the serum iron upon the diurnal variations. G. Hemmeler (*Schweiz. med. Wchnschr.* **73,** *1056 (1943)*) studied a group with high serum iron levels, patients with infectious hepatitis. The average diurnal variation in these patients was from 218 micrograms per cent to 135 micrograms per cent and the maxima and minima occurred at the same time. In a subsequent study Hemmeler (*Helvet. med.*

Acta. **13,** *20 (1946)*) observed that in anemia with low serum iron levels, a diurnal variation of similar relative magnitude occurred (56 micrograms per cent → 39 micrograms per cent). Thus, levels of serum iron do not seem to influence the phenomenon of clinical variation.

L. D. Hamilton, C. J. Gubler, G. E. Cartwright, and M. M. Wintrobe (*Proc. Soc. Exp. Biol. Med.* **75,** *65 (1950)*) restudied this problem because of their interest in iron metabolism. They confirmed that plasma iron was highest during the early morning and fell during the day, reaching its lowest levels during the evening. Because of the similarity of this curve to that of adrenal cortical secretion and because of the effects of stimulation of the adrenal cortex in altering plasma iron levels, the diurnal variation of iron in 2 patients with Addison's disease was measured. It was no different from that in the normal subjects, suggesting that the variations of adrenal activity and serum iron are not causally related but epiphenomena.

J. C. S. Paterson, D. Marrack, and H. S. Wiggins (*Clin. Sci.* **11,** *417 (1952)*) similarly found that patients with Simmonds' and Addison's diseases exhibited normal diurnal variations of serum iron. This was not influenced by administration of ACTH or cortisone.

The net effect of these investigations is to demonstrate the need for considering diurnal variations in investigations which are predicated upon a stable base line. The diurnal variation of iron, per se, is another manifestation of the biologic rhythms which have yet to be unravelled.

THE COMPLEXITY OF THE DENTAL CARIES PROBLEM

Epidemiologic studies of dental caries in various population groups have been reported with increasing frequency since H. T. Dean ("Dental Caries and Fluorine," *p. 5, American Association for the Advancement of Science, Washington, D. C. (1946)*) demonstrated an inverse relationship between the presence of minute amounts of fluorides occurring naturally in public water supplies and the incidence of caries in children. The majority of such studies have been concerned with the influence of fluorides (*Nutrition Reviews* **9,** *21 (1951)*; **10,** *74 (1952)*), and have stimulated current public interest in fluoridation as a public health measure.

For several years, D. M. Hadjimarkos has been conducting an epidemiologic study of dental caries in the state of Oregon, which re-emphasizes the well known but perhaps underemphasized fact that the development and maintenance of healthy, noncarious teeth is dependent upon a complex of nutritional and environmental factors, and not solely upon the fluoride content of the water. This broader view does not imply any reservation about the value of water fluoridation. Hadjimarkos (*J. Pediat.* **48,** *195 (1956)*) has recently reported his findings in the Blue Mountains region of Oregon, where he surveyed the caries experience, as measured in terms of DMF teeth (decayed, missing, filled), among native-born and native-reared white school children 14 to 16 years of age living in 2 counties. The population of these 2 counties has been stable for the past twenty years. Both counties are located east of the Cascade Mountains, in that portion of Oregon which has an arid or semiarid climate, high altitude, and about 140 clear sunshiny days per year. Hadjimarkos has compared the caries experience of these children living in eastern Oregon with similar groups of children, whom he has previously studied and reported on, living in counties west of the Cascade range, in western Oregon where the climate is characterized by high rates of precipitation, low altitude, fertile valleys and thick vegetation.

Statistical analysis of his epidemiologic data shows that children born and reared in regions east of the Cascade range, where there is a higher altitude and more sunshine,

have significantly lower caries prevalence rates than those of children living in regions west of the mountains. Rates of DMF teeth, expressed per child, ranged from 9.0 to 11.3 among 4 counties located east of the Cascades, and from 11.8 to 14.4 among 6 counties situated west of these mountains.

Determination of fluoride content and total hardness of public water supplies, amounts of candy bars and carbonated beverages consumed, and ratio of dentists to population failed to demonstrate any significant differences between the groups in eastern and western Oregon.

Two significant relationships are reported, which Hadjimarkos believes may contribute to the lower incidence of caries among children living in eastern Oregon: (1) an inverse relationship between amount of sunshine available in an area, in combination with altitude, and caries prevalence rates, and (2) a direct relationship between urinary selenium concentration and susceptibility to caries.

Because ultraviolet radiation from the sun stimulates the production of vitamin D by action upon the provitamin located beneath the surface of the skin, and because it is known that the biologically effective ultraviolet radiation may be greatly influenced by atmospheric pollution from smoke, dust, fog, haze, and also by altitude, Hadjimarkos reasons that the children living at the higher altitude in a sunnier climate have fewer dental caries because of their greater exposure to solar ultraviolet radiation during the period of dental calcification.

The direct relationship to urinary selenium concentration recalls previously published observations on animals. Using white rats, M. G. Wheatcroft, J. A. English, and C. A. Schlack (*J. Dent. Res.* **30,** *523 (1951)*) have shown that selenium may make the teeth of rats more susceptible to caries. Though the mechanism of this action is unknown, studies of the various toxic effects of selenium are under way in many laboratories (*Nutrition Reviews* **13,** *254 (1955)*).

This work of Hadjimarkos and similar studies by other conservative epidemiologists demonstrate the importance of the total environment in relation to human health. When a disease is influenced by multiple causes, the total cure or prevention must necessarily be complex. This fact is often not appreciated or emphasized when a specific treatment is being investigated or applied.

CLINICAL STUDIES WITH COBALT-LABELED VITAMIN B_{12}

The recognition of the cobalt moiety of vitamin B_{12} and the ready availability of a labeling agent in radioactive cobalt (Co^{60}) have made possible an analysis of a number of syndromes in which the absorption of vitamin B_{12} may be a significant factor. It has permitted delineation of the role of intrinsic factor and the intestinal mucosa with greater surety than the previous technics which depended upon reticulocytosis. Some of the earlier results and technics were previously reviewed (*Nutrition Reviews* **12,** *4 (1954)*).

R. F. Schilling (*Am. J. Clin. Nutrition* **3,** *45 (1955)*) has demonstrated that, when normal patients are given 1 to 2 micrograms of vitamin B_{12} labeled with Co^{60}, none is excreted in the urine in twenty-four hours. However, when a carrier dose of 1000 micrograms of vitamin B_{12} is injected simultaneously with the ingestion of the labeled vitamin B_{12}, 7 to 21 per cent of the radioactivity is recovered in the urine in twenty-four hours. Twenty-three patients with pernicious anemia were studied in this way and the twenty-four-hour urine excretion was 0 to 2.3 per cent. In 26 patients with histamine-fast achlorhydria, the excretion ranged between 3.2 and 9.6 per cent. This group is particularly hard to evaluate, as some of

these patients may represent early pernicious anemia (*see below*).

M. I. Klayman and L. Brandborg (*New Engl. J. Med.* **253,** *808 (1955)*) used the above method to study the handling of vitamin B_{12} in a variety of syndromes. The range for normal subjects was 10 to 12 per cent urinary excretion in twenty-four hours. Patients with pernicious anemia excreted less than 1 per cent. These workers were unable to diagnose patients with achlorhydria conclusively on the basis of twenty-four-hour urinary excretions in the range of 1 to 2.6 per cent. One patient with achlorhydria excreted no labeled vitamin B_{12}. As it is known that following total gastrectomy it takes two to five years for the development of pernicious anemia (*loc. cit.*), some cases of achlorhydria will inevitably be studied during the phase of depletion of body stores of vitamin B_{12} before clinical disease develops.

The specific defect of pernicious anemia can be distinguished from other megaloblastic anemias. Klayman and Brandborg (*loc. cit.*) were unable to increase the absorption of vitamin B_{12} by giving intrinsic factor to one patient with massive small bowel resection and to one subject with the linitis plastica type of gastric carcinoma. Three other patients with carcinomas of the stomach handled orally administered vitamin B_{12} normally. Similarly, S. T. Callender and J. R. Evans (*Clin. Sci.* **14,** *387 (1955)*) were unable to increase absorption of vitamin B_{12} in a patient with massive resection of the jejunum.

S. Callender, A. Turnbull, and G. Wakisaka (*Clin. Sci.* **13,** *221 (1954)*) examined the excretion of vitamin B_{12} after total gastrectomy by measuring the percentage of labeled vitamin B_{12} in the feces after ingestion of 0.5 micrograms of vitamin B_{12} labeled with Co^{60}. In their control group 14 to 45 per cent appeared in the feces. In 13 patients with pernicious anemia 76 to 100 per cent appeared in the feces. Following total gastrectomy, vitamin B_{12} given orally was excreted in the same fashion as in pernicious anemia. Three of the patients were studied six months after surgery when there was no evidence of anemia. Their excretion of vitamin B_{12} was the same as that in 3 patients examined two and one-half to three years after gastrectomy. This confirms the earlier impressions that the development of megaloblastic anemia takes a few years following total gastrectomy, although the defective absorption of vitamin B_{12} can be demonstrated soon after gastrectomy.

In order to analyze further the quantitative relationships between vitamin B_{12} absorption and intrinsic factor, Callender and Evans (*loc. cit.*) administered cobalt-labeled vitamin B_{12} by mouth in amounts varying from 0.25 to 5.5 micrograms. The absorption was estimated by measuring the fecal radioactivity. They found that the total absorption increased up to 1 microgram per day and then leveled off.

In patients with pernicious anemia the absorption of vitamin B_{12} was proportional to the amount of semipurified intrinsic factor that was given up to a certain level when a plateau was reached. When excessive amounts of intrinsic factor were given (forty times the maximum effective dose) there was a decrease in the amount of vitamin B_{12} absorbed. These workers were unable to achieve greater absorption of vitamin B_{12} than normal in pernicious anemia by giving both vitamin B_{12} and intrinsic factor in excess. It appears, therefore, that there is a fixed limit to the capacity of the intestine to absorb vitamin B_{12}. The decrease in absorption after administration of excessive amounts of intrinsic factor may be only apparent as intrinsic factor has been reported to contain small amounts of vitamin B_{12} (S. J. Baker and D. L. Mollin, *Brit J. Hematol.* **1,** *46 (1955)*).

Callender and Evans utilized the same technics in studying another disease characterized by megaloblastic anemia, idiopathic steatorrhea. One half of these patients had low absorption of vitamin B_{12} which was not corrected by amounts of intrinsic factor

which are effective in pernicious anemia. However, with high doses of intrinsic factor, the absorption of vitamin B_{12} was definitely enhanced.

Another clinical problem which has lent itself to analysis by these methods concerns the etiology of combined system disease without anemia. An unravelling of this syndrome was undertaken by I. M. Arias, L. Apt, and M. Pollycove (*New Engl. J. Med.* **253,** *1005 (1955)*). In their report these workers state that they had determined absorption of vitamin B_{12} by measuring hepatic deposition, fecal elimination and urinary excretion, and that the three methods gave comparable results. They utilized for their studies hepatic deposition of high specific activity cobalt-labeled vitamin B_{12}. With this method they assayed intrinsic factor activity in nonanemic patients with combined system disease who had not received previous therapy. These patients had less than 19 per cent hepatic deposition while the normal control group varied between 40 and 70 per cent. These percentages represent oral dose divided by hepatic deposition after injection times 100.

The gastric juice from these patients with combined system disease did not increase the hepatic deposition of Co^{60}-labeled vitamin B_{12} in pernicious anemia. Conversely, oral absorption of vitamin B_{12} in the test patients was increased by normal gastric juice. Therefore, if absence of intrinsic factor be equated with pernicious anemia, the point has been proven that pernicious anemia can manifest itself as combined system disease without anemia.

Thus, the rapid advances in this segment of hematology have clarified a number of clinical problems. It seems to be proved that the metabolic defect in pernicious anemia is an absence of intrinsic factor and that gastric mucosa is the only source of this factor. The stoichiometric relationship of intrinsic factor and vitamin B_{12} is apparent and an extension of this work requires further purification of intrinsic factor. Some patients with combined system disease have been found to have the same metabolic defect as pernicious anemia patients, demonstrating that anemia is not a *sine qua non* of the combined system disease due to lack of vitamin B_{12}.

THE OBESE CHILD

The report of Fröhlich in 1901 called attention to the correlation of obesity and sexual immaturity with neoplasia in the region of the pituitary gland in a 14 year old boy. Since that time endocrine stigmata have frequently been associated with childhood obesity in lay and medical minds. O. H. Wolff (*Quart. J. Med.* **24,** *109 (1955)*) has done much to remove these stigmata and to relegate consideration of the obese child to its proper perspective.

Beginning with a brief but revealing review of the varied and often conflicting conclusions drawn by casual clinical observations of childhood obesity, Wolff utilizes measurements made on the first 100 children who attended the obesity clinic in the Children's Hospital, Birmingham, England, to clarify the developmental status of the obese child.

His measurements indicate: (1) that the birth weight of the obese child was identical in mean and distribution to the normal distribution of birth weights of children in Britain; (2) that the height of obese children was significantly greater than the average height of children of the same age; (3) that the children who lost weight on a reducing diet failed to increase their height at the expected rate during this period; (4) that puberty (development of nipples and pubic hair growth) began definitely earlier in obese girls than has been reported in nonselected standard groups of girls or in tall girls in the

same environment; and (5) that puberty in obese boys (growth of pubic and axillary hair) began, on the average, approximately one year before it is expected in nonselected groups of boys in the same environment.

From these findings Wolff makes the following deductions: (1) that childhood obesity is usually acquired, although abnormal appetite-regulating mechanisms are not ruled out; (2) that the obesity and the abnormally great height of the obese child are related to overeating and not to endocrine factors, since both height and weight were similarly affected by caloric restriction; (3) that the earlier onset of puberty in the obese child can probably be correlated with the faster growth of these children in other parameters and does not indicate endocrine abnormality. Certainly it does not suggest endocrine insufficiency in any sense.

Wolff interprets his height-weight figures to show that weight reduction in the obese child is not contraindicated because of the suppression of height gain which characteristically accompanies it. He points out that a child who is 100 per cent above standard weight will show only a 20 per cent retardation in growth in height during the twenty months required to reduce the weight to normal. Since these children are usually taller than normal to start with, the slight interference with gain in height during weight reduction need not be regarded as important. In fact, since these obese children enter puberty early, Wolff points out that they probably would cease to grow a year or so earlier than usual. Thus weight reduction may, in the long view, enable them to grow taller than they might otherwise.

These observations and the author's sensible interpretations should be helpful to those who examine and treat the obese child.

WATER AND SALT ABSORPTION IN THE SPRUE SYNDROME

The more obvious absorption defects in the sprue syndrome have been extensively studied, but because of a lack of clinical evidence of disturbances in water and sodium metabolism these factors have not been so well investigated. Some time ago it was noted by M. S. Levy, M. H. Power, and E. J. Kepler (*J. Clin. Endocrinol.* **6,** *607 (1946)*) that a patient with idiopathic steatorrhea gave a false positive response to the Cutler-Power-Wilder water test for adrenal insufficiency. This clue led to studies by E. E. Wollaeger and B. H. Scribner (*see Nutrition Reviews* **10,** *156 (1952)*), who found that patients with the sprue syndrome exhibited nocturnal diuresis of a water load. They claimed that water absorption was delayed only if food was in the intestine, the presumption being that the water was osmotically obligated and absorption was thus delayed.

W. H. Taylor (*Clin. Sci.* **13,** *239 (1954)*) re-examined this problem in 23 patients with idiopathic steatorrhea. He used a standardized water tolerance test in which the water excretion two hours after ingestion of a liter of water was compared in the normal and the experimental groups. These tests were performed two hours after breakfast. There was a significant delay in water excretion in the patients with steatorrhea. The per cent of water excreted was positively correlated with the per cent of fat absorbed, suggesting that the difficulty in water absorption varied in severity concomitant with the defect in fat absorption.

In a subsequent study W. H. Taylor (*Clin. Sci.* **14,** *725 (1955)*) eliminated the possible alterations in water diuresis due to food in the intestine. When the same test was applied to patients in the fasting state the amount of water excreted remained significantly less than in the control group. After water ingestion, both groups showed dilution of serum chloride, presumably as a result of

migration of chloride into the hypochloremic intestinal contents. However, the concentration of blood solids decreased in the normal patients and remained unchanged or increased in 6 of 8 of the patients with steatorrhea. This seems clearly to fix the defect in water diuresis in the sprue syndrome to delayed water absorption. The 2 patients of this group who diluted their blood solids normally had normal water diureses.

G. A. Newsholme and J. M. French (*Clin. Sci.* **13,** *607 (1954)*) have reported their results on the absorption of sodium in the sprue syndrome. They injected 50 microcuries of radioactive sodium chloride ($Na^{24}Cl$) into the second and third parts of the duodenum and measured its absorption into the blood stream by following the counting rate over the hand. Six patients with severe steatorrhea demonstrated a delayed rise in the counting rate. Two patients with less severe disease had normal absorption of sodium. Two of those with severe cases were re-examined after clinical recovery, and the sodium absorption had become normal.

Newsholme and French considered the possibility that previous sodium depletion in patients with steatorrhea altered the apparent sodium absorption by permitting sodium to enter cells more rapidly. However, a normal total exchangeable sodium was demonstrated in one of these patients, tending to rule out this hypothesis. They further demonstrated a delay in radioactive iodine (I^{131}) absorption in one of the patients with steatorrhea.

Thus, the studies cited above further suggest that the sprue syndrome involves multiple defects of absorption and that the term "malabsorption syndrome" truly describes this disease.

ADRENOCORTICAL FUNCTION IN JUVENILE DIABETES

Complex interrelationships of multiple nutritional and endocrine factors in the metabolism of carbohydrates continue to stimulate the interest of many workers. During the past ten years, the introduction of practical laboratory methods which permit reasonably accurate quantitative measurements of steroid products of the adrenal cortex in urine and blood has been closely followed by the appearance in the literature of increasingly refined and elegant studies designed to elucidate the function of the adrenal cortex in carbohydrate metabolism. Each of these studies has answered some questions, while raising others.

That adrenocortical hyperactivity occurs as a part of the body's reaction to diabetic acidosis has been conclusively demonstrated by J. W. McArthur and her co-workers (*J. Clin. Invest.* **31,** *592 (1952)*; **33,** *420, 437 (1954)*; *Nutrition Reviews* **13,** *188 (1955)*). Thirty-six hours after these workers had stopped administering insulin to dogs rendered diabetic by pancreatectomy, they found evidence of adrenocortical hyperfunction, as indicated by alterations in quantities of circulating white blood cell groups and by rising concentrations of corticosteroids in urine, as well as by changes in electrolyte balance characteristic of hyperadrenocorticism. They also noted that the animals became much more sick at the same time as the laboratory signs of adrenocortical overactivity appeared. Several of the animals died rapidly in spite of the institution of appropriate therapy. The onset of all these metabolic aberrations could be hastened by administration of ACTH, and the reaction was ameliorated by adrenalectomy.

The adrenal cortex has also been shown to play a role in the development of the late vascular complications of diabetes. B. Becker and his associates (*Diabetes* **3,** *175 (1954)*; *Nutrition Reviews* **13,** *325 (1955)*) have implicated adrenocortical dysfunction as an etiologic factor in diabetic retinopathy, on

the basis of more active eosinopenic responses to administered ACTH observed among diabetic patients with retinopathy as compared to those without retinopathy.

The functional status of the adrenal cortex in juvenile diabetic patients under satisfactory control has recently been studied by R. Klein, F. A. Weigand, M. Iunes, and L. Greenman (*Pediatrics* **17,** *214* (*1956*)), who report that children with adequately treated diabetes have levels of free adrenocortical steroids in their blood serum which are significantly higher than those found in the blood of normal children.

Three groups of children were studied. A group composed of institutionalized children and children coming to the outpatient clinic for minor problems furnished normal control data. The second group included 79 clinic patients, ranging in age from 4 years 8 months to 15 years 8 months, with known diabetes, who were considered to be in satisfactory metabolic condition. These patients were all receiving measured diets, and sufficient insulin to achieve minimal glycosuria without inducing hypoglycemic reactions. Weight gain and growth had been satisfactory in each case. The third group consisted of 18 juvenile diabetic subjects who were hospitalized for re-education or adjustment of insulin dosage at the time of study.

Venous blood was obtained from each subject between 8 a.m. and 9 a.m., prior to breakfast and before administration of the morning dose of insulin to the diabetic patients. Blood samples were analyzed for content of free corticoids in serum by the method of D. H. Nelson and L. T. Samuels (*J. Clin. Endocrinol.* **12,** *519* (*1952*)), and levels of carbon dioxide, chloride, sodium, potassium, sugar, nonprotein nitrogen, inorganic phosphorus, cholesterol, and circulating eosinophils were also measured. Among the diabetic patients, the first urine specimen voided in the morning was tested for the presence of reducing sugar, acetone and diacetic acid by accepted clinical methods.

The mean value of free corticoid concentration in the serums of the normal children studied was 14 micrograms per 100 ml. of serum, the standard error of the mean being ± 0.89. The mean free corticoid concentration in the serums of the 79 diabetic children studied in the outpatient clinic was 20 micrograms per cent, standard error being 0.98. This mean value is significantly greater ($P < 0.01$) than that of the normal children studied by these workers.

Analysis of their data revealed to Klein and his associates that two groups of diabetic children could be distinguished on the basis of the concentrations of free adrenocorticoids in their serum. The first group included 34 patients, whose serum corticoid levels were less than 18 micrograms per cent. Significantly correlated with a serum corticoid level of less than 18 micrograms per cent were a higher serum carbon dioxide content (average 23.1 milliequivalents (mEq) per liter), and a lower concentration of reducing substances and absence of acetone in the urine specimens. In contrast, 45 patients whose serum corticoid levels ranged from 19 to 46 micrograms per cent had lower serum carbon dioxide contents (average 21.8 mEq per liter), and spilled greater quantities of sugar in their urine. In addition, there were 11 cases of acetonuria, plus 3 instances of urinary diacetic acid, in these patients.

Further subdivision of these two groups of patients on the basis of concentrations of serum corticoids emphasizes the existence of an inverse relationship between corticoid level and carbon dioxide content of the serum, and a direct relationship between serum corticoid level and the concentration of reducing substances, acetone and diacetic acid present in the urine. No significant differences could be found between the two groups divided on the basis of serum corticoid level with respect to age, sex, duration of diabetes, insulin dosage, or any of the other studies performed on venous blood.

Diurnal variations in levels of free corti-

coids in the serum were measured in the 18 hospitalized children with diabetes, and were normal. These same 18 children showed normal changes in free serum corticoid levels following administration of ACTH (10 mg. per square meter of body surface area, given intravenously) or cortisone (60 mg. per square meter of body surface area, given orally). Klein and his colleagues therefore believe that the elevated levels of free corticoids in the serums of their diabetic patients are not due to any abnormalities in the metabolic utilization or breakdown of adrenal corticoids once these have been released into the blood stream.

The authors select three possible interpretations of their own data for brief discussion: (1) An increase in adrenocortical activity (primary, or secondary to hypothalamicopituitary activity) may be a primary part of diabetes mellitus in the young patient; similar investigations of serum corticoid levels in prediabetic children would help to establish or disprove this concept. (2) The increased availability of acetyl-coenzyme A, and its concurrent diminished utilization by other tissues of diabetic patients (*Nutrition Reviews* **13,** *74 (1955)*), may lead to overproduction of steroid hormones by the adrenal cortex; the lack of correlation of corticoid level with cholesterol content of the serum argues against this interpretation. (3) The inference can be drawn that a child with diabetes who has elevation of adrenocorticoid concentration in his serum is under constant stress because of his diabetes and its treatment.

The correlation of serum corticoid levels in diabetic children with criteria ordinarily used to estimate the "degree of control" of the diabetes, *i.e.*, urinary reducing substances and acetone, and evidences of acidosis, suggests to the authors that children in poor control are under constant stress. However, they also consider an alternative possibility that poor control may be the result of more severe disease rather than of the therapeutic program prescribed or the faithfulness with which the regimen is carried out by the patient and his family.

The studies which have been summarized strongly suggest that adrenocortical steroids exert important effects in the metabolism of carbohydrates. Furthermore, the derangement of normal carbohydrate metabolism described clinically as diabetes mellitus is characterized not only by an insufficiency of endogenous insulin, but also by hypersecretion of the adrenal cortices, at least in children with the disease. Adrenocortical hyperactivity is even further increased during the response to hypoinsulinism described clinically as diabetic acidosis. The ease with which the clinician can "regulate" the diabetes of an individual patient by means of dietary and insulin therapy may be governed, at least in part, by the adrenocortical functional status of the patient. Onset of vascular degenerative changes in patients with diabetes may relate to the severity of the general endocrine imbalance present, as a part of which adrenocortical hyperactivity may prove to be at least as important as hypoinsulinism or dietary abnormalities. Additional studies which utilize methods similar to those employed by McArthur and her co-workers and by Klein and his colleagues should help to clarify the role of adrenal corticosteroids in normal carbohydrate metabolism, and also should improve the ability of clinicians to treat physiologically the most common of all diseases due to endocrine imbalance, diabetes mellitus.

METABOLIC EFFECTS OF DIHYDROTACHYSTEROL

In order to delineate the actions of dihydrotachysterol (antitetanic preparation No. 10; A.T. 10) on calcium and phosphorus metabolism, P. D. Saville and co-workers (*Clin. Sci.* **14,** *489 (1955)*) have studied metabolic balance in 6 patients with resistant

rickets. The serum calcium was normal in these patients, but the serum phosphorus was 3.0 mg. per cent or less. This value is quite low when it is compared to the normal value of 4.5 to 5.0 mg. per cent in the growing child.

A.T. 10 was given in doses of 1.25 mg. to 10 mg. daily. In all cases this resulted in a rapid decrease in fecal calcium and phosphorus. In 4 of the patients there was no change in urinary calcium or phosphorus, so that a considerable positive balance was achieved. In one patient with healing rickets, urinary calcium and phosphorus excretion increased so that the net positive balance was small, and in one other patient excessive urinary loss of calcium and phosphorus led to negative balances.

Following the initial period of study with A.T. 10, 1 g. of phosphorus (as sodium acid phosphate) was added to the regimen. This led to a rise in serum phosphorus to levels of 5 mg. per cent, a slight drop in serum calcium and a marked decrease in urine calcium. In no case did the addition of sodium phosphate increase the fecal calcium, so that calcium balance became more positive in every instance.

These authors review previous pertinent experimental data and suggest that the effects of A.T. 10 and vitamin D are, in part, dependent upon the status of bone metabolism. Thus, in the calcium-depleted subject, calciferol will increase intestinal absorption of calcium and phosphorus while increasing renal tubular reabsorption of phosphate. In the normal individual, however, calciferol increases the urinary loss of phosphorus without altering the serum levels, so that renal tubular reabsorption must have decreased. Previous comparative studies of A.T. 10 and calciferol ignored this point. The authors conclude that A.T. 10 and vitamin D are alike in their effect upon calcium and phosphorus metabolism.

The treatment of resistant rickets with vitamin D has not been satisfactory, although positive calcium balance may be attained. Usually, despite these measures, the serum phosphorus remains below 4 mg. per cent. If the childhood level of serum phosphorus of 5 mg. per cent is necessary for optimal bone growth, then measures to achieve this would be of practical importance.

The studies reported here demonstrate conclusively that the addition of phosphorus to the diet increases the positive calcium balance and raises the serum phosphorus. Thus, the antirachitic effect of A.T. 10 was increased by this measure. As the effects of A.T. 10 or vitamin D cannot be increased by raising the dose because of higher urinary excretion of calcium, this addition of phosphorus to the diet constitutes a valuable measure in the treatment of resistant rickets.

PHOSPHATE TRANSFER IN THE DIGESTIVE TRACT

An exchange of minerals between the gastrointestinal contents and the blood has long been recognized as a normal physiologic process. Such an exchange provides an appropriate buffer for digestive secretions and probably also serves in other important ways to facilitate normal functioning of the digestive system. Secreted phosphorus (endogenous phosphorus) in intestinal contents in the rat was estimated to be equal to or exceeded by that supplied by the feed, in studies conducted many years ago with the use of an inert material (ferric oxide) as a reference or tracer substance (O. Bergeim, *J. Biol. Chem.* **70,** *51 (1926)*). This so-called "internal circulation of phosphorus" has recently been studied in swine by the use of radioactive phosphorus (P^{32}) as a reference substance (A. H. Smith, M. Kleiber, A. L. Black, and J. R. Luick, *J. Nutrition* **57,** *497 (1955)*).

Swine 2, 4 and 8 months of age, representing 5, 20 and 50 per cent of mature body weight respectively, received a single in-

jection into the marginal ear vein of a buffered isotonic solution containing radioactive phosphate. The animals were slaughtered one to forty-eight hours following injection. Digestive tract tissue and contents were sampled as follows: stomach, small intestine (at the midpoint and posterior to the entry of bile and pancreatic juice), cecum, colon and rectum. Dry matter, phosphorus and radioactivity determinations were made on all samples. The radioactivity analyses were expressed as specific activity, that is, microcuries of P^{32} per milligram of total phosphorus. This value divided by the millicurie of P^{32} injected per kilogram of body weight served as the standardized specific activity.

The highest specific activity of digestive tract contents was found in the small intestine six hours after dosage. At this time the specific activity in intestinal contents was four to five times as great as that found in stomach contents. Since intestinal capacity exceeds that of the stomach by about 15 per cent, and since total phosphorus in intestinal contents exceeded that in stomach contents by only about 20 per cent, the results indicated that a much larger amount of endogenous phosphorus entered the intestine than the stomach. This observation is in agreement with the radioactive phosphate studies in rats where intestinal contents were observed to contain about five times as much endogenous phosphorus as stomach contents (K. Kjerulf-Jensen, *Acta physiol. Scandinav.* **3,** *1 (1941/42)*).

The contents of the intestine posterior to the small intestine exhibited decreased specific radioactivity. Since this was true within one hour after injection of the isotope and also after forty-eight hours, it indicated that (1) secretion of endogenous phosphorus into cecum, colon and rectum was small and that (2) there must have been a much greater removal (reabsorption) of the endogenous phosphorus than of the total phosphorus as the contents moved toward the distal end of the digestive tract.

Lesser quantities of isotope were consistently found in the intestinal contents of the younger than of the older animals. This presumably is an expression of the higher competition for phosphorus for bone formation in the younger animals, which would tend to reduce circulatory phosphate secretion into the intestine. This was confirmed in the observation that the single dose of tracer disappeared more rapidly from the circulation in the young animals (2 months old). Taking into account the more rapid loss of radioactivity from the circulation in the young animals, the data definitely establish that aging actually sharply decreased the secretion of endogenous phosphorus. For example, when the specific activity of the material in the colon and rectum relative to that of plasma inorganic phosphorus was calculated, the relative specific activities of these materials in 2 month old animals, six and twenty-four hours after injection of the isotope, were almost one-hundred times as high as the comparable samples in 8 month old animals.

The distribution of radioactivity among the tissues of the gastrointestinal tract was somewhat different from that of the contents. Maximum specific activity was found in tissue of the cecum and colon and this maximal activity occurred before there was any appreciable activity in the contents. Aging was observed to decrease the rate of uptake of radiophosphorus by all the gastrointestinal tissues.

Similar studies were also conducted with sheep, an animal in which the digestive process is characterized by prolonged storage of ingested material in the rumen where microbial activity is extensive (A. H. Smith, M. Kleiber, A. L. Black, and C. F. Baxter, *J. Nutrition* **57,** *507 (1955)*). Three age groups were included, 1, 4 and 10 month old sheep. Standard specific activities for digestive tract contents and tissues were calculated from the analytic results following a single injection of radioactive phosphorus. The results indicated that sheep absorbed a much greater amount

of phosphorus from the digestive tract than swine. Although the phosphorus concentration of omasal contents in 10 month old sheep was four times that in the 8 month old swine stomach, the sheep rectal contents had only half the phosphorus concentration observed in swine.

The secretion of phosphate into the contents of the digestive tract was found to be much more extensive in sheep than in swine. These observations are of interest when it is remembered that the dietary requirement for phosphorus is twice as high in swine as it is in sheep. In sheep the specific activities of the gastrointestinal contents shortly after tracer administration indicated that the major part of the endogenous phosphorus entered the digestive tract in the rumen, contrasted with the small intestine as the major secretion site in swine.

Based on calculated salivary flow and phosphorus content of saliva, it was estimated that about one half of the phosphorus entering the digestive contents in the rumen must have entered by direct passage through the rumen wall.

The high radioactivity of the abomasal contents suggested a secretion of endogenous phosphorus into this organ, while secreted phosphorus found in the omasum was small. Very little radioactivity was noted in the intestines of sheep shortly after injection of the active material, although faint activity was detectable also in the cecal and colonic contents, suggesting some secretory activity at these lower levels.

The high secretory activity of the upper region of the digestive system with respect to phosphate in sheep can perhaps logically be explained as a normal physiologic process in which the phosphate serves to buffer the volatile fatty acids that are formed in microbial fermentation in the rumen. Studies with dairy cattle indicated this species behaved very much like sheep with respect to gastrointestinal phosphate transfer (A. H. Smith, M. Kleiber, A. L. Black, and G. P. Lofgreen, *J. Nutrition* **58,** *95* (*1956*)).

Although the tracer technic as applied by Smith and his associates has provided valuable confirmatory information as to active phosphorus secretion into the digestive system, some of the interpretations regarding rate of secretion of phosphorus at various levels in the digestive system of sheep must be taken with some reservations. For example, these workers calculated that the rumen of their 10 month old sheep contained 8.65 g. of phosphorus. This calculation was based on the estimate that the rumen contents represented 15 per cent of the live weight of the animal. Since an estimate of secretion rate of phosphorus into rumen was based on an estimate of total phosphorus present at any given time, it would have been desirable to obtain a more exact measure of rumen contents.

LIVER FACTORS FOR CHICK GROWTH

Although the identification of vitamin B_{12} in 1948 demonstrated that it was one of the essential factors for chicks present in animal products, there have been numerous reports since that time dealing with other unidentified factors required for the maximum growth of chicks. These have been reviewed (*Nutrition Reviews* **14,** *75* (*1956*)). Substantial progress in the isolation of new factors and the demonstration that they are not identical with known vitamins have been made and presented by H. M. Edwards, D. Chin, L. C. Norris, and G. F. Heuser (*J. Nutrition* **57,** *329* (*1955*)).

The studies were done using a purified ration containing repeatedly washed casein supplemented with glycine, arginine and methionine as the source of protein. All of the known mineral elements required by the chick were supplied, as well as choline, niacin, calcium pantothenate, alpha-tocopherol, thiamine, riboflavin, pyridoxine,

folacin, biotin, 2-methyl-1,4-naphthoquinone, vitamin B_{12} and vitamins A and D. Glucose and 3 per cent of cellophane and of hydrogenated fat completed the ration. Day-old chicks fed this ration weighed about 280 g. in four weeks and growth was not increased by increasing the amounts of vitamins or trace elements present in the ration. The addition of 5 per cent of defatted dried liver or 4.5 per cent of fish solubles increased the growth about 20 per cent to 335 g. in four weeks. Larger amounts of these given singly did not improve the gain, but the combination of the two together increased the gain to between 30 and 40 per cent above that on the basal diet. Apparently these substances contained distinct factors each of which gave a growth stimulus.

A study of various fractions prepared from liver (supplied by the Wilson Laboratories, Chicago) was made. The active material was present in a water or 70 per cent ethanol extract of liver whereas the residue was relatively inactive. Approximately 1.5 per cent of the ethanol extract gave a maximum response, although the resulting growth was slightly inferior to that obtained with whole liver. Extraction with phenol apparently removed the active factor completely and reduced the solids of the liver fraction by 39 per cent and the ash content by 60 per cent. The phenol-soluble extract was further fractionated by an 8-plate countercurrent distribution procedure using phenol and n-butanol in a ratio of 1:1 in the upper layer and 3 per cent acetic acid (pH 2.5) in the lower layer. When the fractions from the various tubes were fed at levels equivalent to 2 per cent of the original liver extract, it appeared that two separate materials were present. The first was concentrated in tubes 1 to 3 and appeared to be about 74 per cent soluble in the phenol-butanol layer. The other was largely concentrated in tubes 7 and 8 and appeared to be 98 per cent soluble in the aqueous layer. Practically all of the inorganic matter was also concentrated in these two tubes, thus suggesting that it may have been inorganic in nature. A. B. Morrison, M. L. Scott, and L. C. Norris (*Poultry Sci.* **34,** *738 (1955)*) have previously presented evidence, along with others (*Nutrition Reviews* **14,** *51 (1956)*), that unidentified growth-promoting minerals exist. On the other hand, very little inorganic material was present with the phenol-butanol-soluble factor. This appears organic in nature and was apparently concentrated about 130 times in the most active tube.

The studies on the activity of the liver fractions were done with the diet still deficient in the fish solubles factor. Hence, the relationship of the factors in fish solubles to those in liver is not clear, although the work on the crude materials obviously indicates that they are distinct. It is perhaps somewhat strange that growth responses nearly equal to the original liver extract were obtained with either of the fractions separated by the countercurrent distribution. One might have expected minimal growth responses with either alone and growth equivalent to the liver extract only when they were combined. In view of the multiple forms of some of the vitamins now known, such as pantothenic acid, pyridoxine and vitamin B_{12}, data of the kind presented are not conclusive as to the occurrence of multiple growth factors. The results do indicate considerable progress toward the identification of presently unidentified growth factors.

ANTIBIOTICS AND GUINEA PIGS

Most reports on the supplementation of animal feeds with antibiotics have stressed the growth-promoting response resulting therefrom (*Nutrition Reviews* **12,** *108, 166 (1954)*). All animals so far studied, with the exception of the guinea pig, show either an

improvement in growth or remain unaffected. The guinea pig appears to be an exception in that the addition of antibiotics to the diet in many cases produces death of the animal within a short while.

The sensitivity of the guinea pig to antibiotics was suggested in a number of bacteriologic studies. In a study of two spirochetal infections in experimental animals, it was observed that when normal guinea pigs weighing between 260 and 300 g. were given 3 mg. of aureomycin subcutaneously two times a day, the animals died within seven to ten days (F. R. Heilman, *Proc. Staff Meetings Mayo Clinic* **23,** *569 (1948)*). For several days prior to death, the abdomen of the guinea pig was distended, and, on autopsy, the large intestine contained an abnormal amount of bulky, soft feces.

During an epidemic of pneumonia in their animal colony, C. M. Ambrus, C. N. Sideri, G. C. Johnson, and J. W. E. Harrisson (*Antibiotics and Chemotherapy* **2,** *521 (1952)*) treated guinea pigs with 10,000 units of sodium penicillin G per kilogram of body weight per day. All the guinea pigs receiving the antibiotic parenterally died within one week, while many of the untreated guinea pigs recovered. Aureomycin in a dosage of 10 mg. per kilogram of body weight per day was also toxic. These workers observed no significant anatomic or histologic changes in the guinea pigs which died.

The toxicity of penicillin for guinea pigs was also noted by P. de Somer, H. Van de Voorde, H. Eyssen, and P. Van Dijck (*Antibiotics and Chemotherapy* **5,** *463 (1955)*). Intraperitoneal injection of 5000 units of potassium penicillin G produced death of 60 to 70 per cent of the young (250 to 500 g.) guinea pigs. Increasing the dose to 100,000 units increased mortality to 90 per cent but did not shorten the forty-eight-hour period intervening before death. These workers claimed that the animals that died after penicillin treatment showed a disappearance of the normal gram-positive aerobic intestinal flora with an overgrowth of gram-negative bacteria which were primarily *Escherichia coli* var. *intermedius*. Injection of a sterile filtrate derived from this bacterium produced the same effects as penicillin. Aureomycin and bacitracin had the same effect on the guinea pig as penicillin.

A good deal of the work on the toxicity of antibiotics for guinea pigs has been done by P. Roine, T. Ettala, A. Raitio, and U. Vartiovaara (*Brit. J. Nutrition* **9,** *181 (1955)*) at the University of Helsinki. They noted that on a stock ration young guinea pigs started losing weight on the second day after supplementation of the diet with 100 mg. of aureomycin per kilogram of diet. Most of the animals were dead within ten days. Those that survived increased slightly in weight but died within five to six weeks. Older animals appeared more sensitive to the aureomycin in that they lost an average of 23 g. a day from the first day of supplementation and died within two weeks. Results similar to the preceding were secured when a purified diet was used.

Loss of weight and death of the guinea pigs resulted when the level of aureomycin in the diet was as low as 25 mg. per kilogram. On the basis of food intake, it was estimated that as little as 0.1 mg. of aureomycin given by mouth was enough to produce disturbances in the animal. This amount of antibiotic could be consumed over a period of days and was sufficient to cause a loss of appetite. The total amount of aureomycin consumed before death intervened ranged from 1 to 3 mg. Approximately 5 per cent of the animals survived for indefinite periods on the aureomycin-supplemented diet; they were apparently immune to the antibiotic.

When aureomycin was injected, it was less toxic than when given in the diet. These Finnish workers measured the food consumption of one group of animals receiving the antibiotic-supplemented diet and then injected an equivalent amount of aureomycin into guinea pigs receiving the basal stock diet. Under these circumstances all the animals receiving the antibiotic-supple-

mented diet died in seven days, whereas those receiving the aureomycin subcutaneously continued to grow at a normal rate. The injection of larger amounts of aureomycin produced death. It was suggested that under such circumstances the antibiotic appeared in the intestinal tract in such concentration that it produced the same effect as the orally ingested antibiotic.

The antibiotic-fed animals showing signs of toxicity stopped eating and drinking. They also had distended abdomens which were primarily due to an enlarged cecum. The body temperature was markedly reduced, the liver showed pale, gray foci of necrosis and was frequently darker in color than normal. The blood showed an increase of neutrophils and a corresponding decrease in lymphocytes. There was, however, no definite monocytosis.

Since the aureomycin was more toxic when fed than when injected, changes in the gastrointestinal tract were looked for. Bacteriologic examination indicated that the aureomycin-supplemented guinea pigs showed a marked increase in *Sarcina* and *Listeria* cells, with a reduction in most other types of bacteria normally found in the cecum. The presence of large numbers of *Listeria* organisms suggested that the guinea pigs were dying from an infection produced by these bacteria.

Injection of a saline suspension of the isolated *Listeria* organisms into guinea pigs produced signs similar to those described above. The primary difference between the guinea pigs inoculated with bacteria and those fed the aureomycin was in the spleen. In the inoculated guinea pigs, the spleen was large and dark purple.

The disease which developed in the antibiotic-fed guinea pigs was reported to be the same as that described by E. G. D. Murray, R. A. Webb, and M. B. R. Swann (*J. Path. Bact.* **29,** *407 (1926)*). Most of the studies of the latter workers were restricted to rabbits, in which the disease was observed especially in the early spring and autumn when fresh food was scarce or "became rank." They reported that careful feeding of the diseased animals produced recovery. The symptoms observed differed from those in the guinea pig in that the appetite of the rabbits was "in no way impaired." On autopsy, all veins in the body were engorged with blood, while the arteries were empty. The subcutaneous tissue was very edematous and jelly-like. The distention of the belly in the rabbits was due to an accumulation of ascitic fluid.

It would appear that terramycin is almost as toxic for guinea pigs as aureomycin. Streptomycin is slightly less toxic, whereas penicillin and chloromycetin have no effect on the growth rate (P. Roine, *Svensk Kem. Tidskr.* **66,** *143 (1954)*).

If the toxicity of the various antibiotics for the guinea pig involves an overgrowth of *Listeria* organisms, then these bacteria vary in their reaction to terramycin. Roine (*loc. cit.*) reported terramycin to be as toxic to guinea pigs as aureomycin, while D. Pohlmann and W. Boese (*Antibiotics Annual, p. 911, Medical Encyclopedia Inc., New York, 1954–55*) found terramycin but not aureomycin effective in treating human cases of *Listeria monocytogenes* meningitis and in *in vitro* studies with the isolated organism.

The work of Roine points out the fact that the intestinal flora may be modified in certain cases by antibiotics in such a way that a harmful effect is produced. This may be brought about by the selective stimulation of harmful bacteria which are normally present in such small numbers that they have no effect upon the animal under ordinary circumstances. There is a possibility that guinea pigs in other laboratories may harbor no organisms comparable to the *Listeria*. Under these circumstances, the addition of aureomycin to the diet may have no effect on growth. Such a possibility may explain the ability of Hogan to use aureomycin in the control of enteritis

in guinea pigs over a number of years (T. Jukes, *Antibiotics in Nutrition, Medical Encyclopedia, New York (1955)*).

On the basis of our present knowledge, it would appear that the action of dietary antibiotics in stimulating, depressing, or not influencing the growth of an animal depends to a large extent upon the microorganisms present in the intestinal tract of the animal or in its immediate environment. The work of Roine and co-workers suggests that guinea pigs harboring only a few *Listeria* organisms can grow at a fairly good rate until something such as aureomycin disturbs the microbial balance, resulting in an overgrowth of *Listeria*.

XANTHURENIC ACID AND DIABETES IN RATS

Xanthurenic acid is well known to be an abnormal metabolite of tryptophan which is produced in the pyridoxine-deficient animal. Y. Kotake (*J. Vitaminology (Japan)* **1**, *No. 2, 73 (1955)*) has summarized a series of experiments carried out by him and his associates which indicate that xanthurenic acid may cause diabetes in rats and that it may be a factor which is relevant in diabetes in man as well.

When relatively large amounts of tryptophan (0.1 g.) and the sodium salts of various fatty acids (0.4 g.) were both administered to rats, the excretion of xanthurenic acid was found to be from three to six times as great as when tryptophan was given alone. Sodium propionate, butyrate, palmitate, oleate, and various other salts of fatty acids were about equally effective. The administration of pyridoxine decreased but did not eliminate the excretion of xanthurenic acid in the urine. Apparently complete and normal metabolism of tryptophan was not possible under the experimental conditions in the presence of large amounts of fat and tryptophan.

Within an hour after administration of the xanthurenic acid and fatty acid, a considerable rise in blood sugar was noted, and after six days of treatment the blood sugar level gradually rose to approximately 180 mg. per cent, with the appearance of sugar in the urine. Similar results were obtained by the intraperitoneal injection of xanthurenic acid, 120 mg. per kilogram of body weight. These animals were receiving a purified diet containing 2 per cent of yeast as vitamin source, 22 per cent casein, 10 per cent butterfat, a salt mixture, starch, and agar-agar. Examination of the pancreas of animals which had received the above treatment (tryptophan plus fatty acid) for forty-eight hours showed a remarkable decrease in the stainability of the beta cells with hematoxylin, vacuole formation, and nuclear pyknosis. No changes in the alpha cells were noted.

A group of animals was fed a diet rich in butterfat (35 per cent), and casein (25 per cent), for 240 days. In order to raise the excretion of xanthurenic acid, 10 mg. of tryptophan was added to the food daily for the first thirty days of the experiment. The vitamin source of the diet is not reported but was presumably supplied by 2 per cent of yeast as in the previous experiments. These animals excreted 2 to 3 mg. of xanthurenic acid per day. The animals gained steadily for about 170 days and attained a maximum weight of 325 g. Normal rats of the strain used are said seldom to exceed 250 to 300 g. The blood sugar levels also gradually rose nearly parallel to the weight gain, and reached about 180 mg. per cent in 160 to 170 days, accompanied by glucosuria. The animals then lost weight and died, although the hyperglycemia was maintained.

In another study the animals were fed a pyridoxine-deficient diet containing 20 per cent casein and 2 per cent fat to which thiamine, riboflavin, inositol, choline and

para-aminobenzoic acid were added. (Pantothenic acid is not mentioned; perhaps this is a printing error). On the twentieth day, 10 mg. of tryptophan were added to the diet daily and this was continued for sixty days. Immediately upon the addition of the tryptophan the growth curve declined and the blood sugar rose. In some animals the blood sugar values fluctuated markedly, but with few exceptions they were in the hyperglycemic range and in some cases above 400 mg. per cent. Xanthurenic acid excretion was usually from 4 to 7 mg. per day. Histologic examination of the pancreas tissue showed edema of the islets and noticeable changes, as described above, in the beta cells.

The urine from 11 diabetic patients and from control subjects was treated with mercuric acetate, the precipitate decomposed with hydrogen sulfide, filtered, and the filtrate concentrated. Paper chromatography revealed the presence of xanthurenic acid and kynurenine in the urine of diabetic subjects but only kynurenine in the urine of control subjects. Kotake states that in an untreated patient there was considerable xanthurenic acid, and that the amount gradually disappeared during the course of treatment (type of treatment not described). Urine from rats made diabetic by the tryptophan-sodium butyrate treatment and similar animals treated with insulin was also examined by paper chromatography. The spot corresponding to xanthurenic acid was observed to be weaker and smaller in the animals receiving insulin but was not abolished by insulin. Quantitative measurement of the xanthurenic acid in the urine of these animals also showed that although the amount could be decreased by insulin, the excretion remained higher than in animals which received only tryptophan without fatty acids.

The author considered the possibility that large amounts of fatty acid might impair liver function, and tested the effect of methionine administration upon the excretion of xanthurenic acid. Methionine decreased the xanthurenic acid excretion more effectively than pyridoxine, and the combination of the two substances nearly abolished the xanthurenic acid in the urine. The data thus suggest that, in the diets used, the pyridoxine and methionine were limiting when the added stress of tryptophan and excess fatty acid was present, and under such conditions an excess of xanthurenic acid is formed. Kotake points out that xanthurenic acid has a structure similar to oxine, which K. Okamoto (*Jap. J. Endocrinol.* **25,** *32 (1949)*) and I. Kadota (*J. Lab. Clin. Med.* **35,** *568 (1950)*) have shown to be a diabetogenic agent in rats. Kotake points out the well known association of diabetes with ingestion of good food, high in protein and fat, and the decreased incidence which occurs during wartime with restriction of national food supplies. Thus, he feels that these studies can be logically equated with situations conducive to the development of diabetes in human beings. Confirmation of these results both in human beings and in animals will be awaited with considerable interest.

METABOLISM OF ASCORBIC ACID

The metabolism of vitamins is important from both practical and theoretic standpoints. Many tests for vitamin nutrition are based on the excretion of the vitamin or its metabolite following a test dose. If the tissues contain adequate stores of the vitamin one would expect that only a small proportion of the administered dose would be retained. The extra quantity might be excreted as such in the urine or it could be altered metabolically and the metabolite excreted. In order to carry out the test

and evaluate the results it is necessary to know how the body treats the particular vitamin in question.

Many of the water-soluble vitamins are excreted in large part unchanged in the urine. It has been found that normal women excrete approximately 20 per cent of a 1 mg. test dose of thiamine in the urine in twenty-four hours (H. L. Mason and R. D. Williams, *J. Clin. Invest.* **21,** *247 (1942)*). Adult human subjects with daily riboflavin intakes of 3.55 mg. excreted approximately one-half this amount in the urine daily (M. K. Horwitt, C. C. Harvey, O. W. Hills, and E. Liebert, *J. Nutrition* **41,** *247 (1950)*). Similar results have been obtained with pantothenic acid. Human subjects excreted in the urine approximately 60 per cent of a daily intake of 4.3 mg. (H. G. Oldham, M. V. Davis, and L. J. Roberts, *Ibid.* **32,** *163 (1946)*).

Certain other vitamins are altered in the body and various products appear in the urine. Administered niacin is excreted in the urine in large part as N-methylniacinamide (J. W. Huff and W. A. Perlzweig, *J. Biol. Chem.* **150,** *395 (1943)*) and the 6-pyridone of N-methylniacinamide (W. E. Knox and W. I. Grossman, *Ibid.* **168,** *363 (1947)*). Pyridoxine and pyridoxal, forms of vitamin B_6, are oxidized to pyridoxic acid, which is the chief urinary end product of this vitamin (B. C. Johnson, T. S. Hamilton, and H. H. Mitchell, *Ibid.* **158,** *619 (1945)*).

Other vitamins appear to undergo more drastic metabolic degradation and no urinary end products are found. For example, choline is destroyed in the body with considerable speed and only traces are found in the urine. It is probably oxidized to carbon dioxide and water (B. C. Johnson, T. S. Hamilton, and H. H. Mitchell, *J. Biol. Chem.* **159,** *5 (1945)*).

The metabolic disposal of ascorbic acid has not been extensively studied. In the well known loading test up to 50 per cent of a test dose is excreted as such in the urine by subjects saturated with the vitamin. The fate of the remainder has not been elucidated. J. J. Burns, P. G. Dayton, and S. Schulenberg (*J. Biol. Chem.* **218,** *15 (1956)*) have studied the metabolism of ascorbic acid in guinea pigs. The animals were injected with uniformly C^{14}-labeled ascorbic acid or carboxyl-labeled ascorbic acid, and expired carbon dioxide and urine were collected. The radioactivity of urinary ascorbic acid, dehydroascorbic acid, diketogulonic acid, and oxalic acid was determined in various experiments.

It was found that following the injection of either carboxyl-labeled ascorbic acid or uniformly labeled ascorbic acid more than 30 per cent of the radioactivity was recovered in the expired carbon dioxide in a subsequent forty-eight-hour period. During the same period approximately 10 per cent of the injected dose of C^{14} was recovered in the urine. Fractionation of the urine excreted by guinea pigs injected with the uniformly labeled ascorbic acid revealed that approximately 30 per cent of its radioactivity was present in ascorbic acid and about 20 per cent in diketogulonic acid. About 9 per cent of the urinary C^{14} was present in oxalic acid while less than 1 per cent of the total urinary activity was recovered in dehydroascorbic acid. In animals injected with carboxyl-labeled ascorbic acid there was a greater percentage of radioactive oxalic acid. The other findings were similar to those obtained with the uniformly labeled vitamin.

In other experiments guinea pigs were injected with carboxyl-labeled ascorbic acid and the total C^{14} excreted in the urine and as respiratory carbon dioxide was determined daily for six days. The percentage of the administered dose of C^{14} remaining in the animal was then plotted as a function of time after the injection. This permitted calculation of the half-life of ascorbic acid in the guinea pig, that is, the time required for removal of one half of the animal's tissue ascorbic acid and its replacement with

exogenous vitamin. The values were found to range from two to six days, with an average of four days.

These experimental results demonstrate that in the guinea pig the major pathway for ascorbic acid metabolism is complete oxidation to carbon dioxide. The relatively short half-life of ascorbic acid in the guinea pig may be a factor in explaining the rapidity with which the signs of scurvy will appear when this animal is deprived of ascorbic acid. In this connection it has been shown that in man the administration of radioactive ascorbic acid does not lead to the expiration of radioactive carbon dioxide, but that the major excretory pathway is via the urine (L. Hellman and J. J. Burns, *Fed. Proc.* **14,** *225 (1955)*). The half-life of ascorbic acid in man was found to be about eighteen days.

These studies also emphasize that among the factors which must be considered as contributing to the development of vitamin deficiency diseases one must include factors which might affect the rate of metabolic degradation of the vitamin. It is conceivable that one vitamin could function in a coenzyme concerned in the degradation of another vitamin. Such a situation might explain the well known fact that when a multiple-vitamin deficiency is treated with a single vitamin one frequently precipitates the deficiency signs of another vitamin.

PHENOTHIAZINE AND THYROID FUNCTION

Phenothiazine (thiodiphenylamine) N. F. (green) is one of the most useful and widely employed drugs for the control of external parasites in farm animals. It is often used as a regular constituent of the ration of livestock as a preventive.

Over the past several years a number of papers have appeared indicating an effect of this anthelmintic on thyroid function. The reported antithyroid action of this substance could serve as an explanation, at least in part, for its beneficial effects in improving weight gain when included in the ration of livestock. More generally the improvement in livestock gains has been considered to be associated with the anthelmintic properties of phenothiazine N. F. (green). The antithyroid action of this anthelmintic, as evidenced by reduced uptake of I^{131} (radioactive iodine), was observed in rats fed a ration in which the drug had been included accidentally (R. V. Talmage, H. Nachimson, L. Kraintz, and J. A. Green, *Science* **118,** *191 (1953)*).

Following this observation these investigators conducted controlled experiments in which 150- to 200-gram rats were fed an iodine-low diet supplemented with 0.1 per cent of commercial phenothiazine. About 10 per cent of a single dose of radioactive iodine was observed to be taken up by the thyroid gland in control animals while animals receiving the drug exhibited an uptake of only 0.9 to 1.9 per cent after one to three days on the experimental diet. The effect of the drug was dramatic, and suppression of radio-iodine uptake by the thyroid gland was evident within two hours after the ration containing the drug was offered. The authors noted that the antithyroid action of the drug was of the same order as that observed by R. W. Rawson and co-workers (*J. Pharmacol. Exp. Therap.* **93,** *240 (1948)*) when 0.1 per cent of thiouracil was fed in the ration to rats for sixteen days.

Following the initial observation on the antithyroid action of phenothiazine in rats, it was established that oral administration of the drug produced a similar inhibition of radio-iodine uptake by the thyroid in such stock animals as burros, sheep, hogs, cattle and chickens (R. V. Talmage, B. F. Trum, R. A. Monroe, and J. H. Rust, *J. Am. Vet. Med. Assn.* **123,** *328 (1953)*; Talmage, Monroe, and C. L. Comar, *J. Animal Sci.* **13,** *480 (1954)*).

In the course of these studies various commercial preparations of the drug were used, including one purified form, and the results indicated that the factor or factors responsible for inhibition of radio-iodine uptake were confined to impurities in the drug. Phenothiazine N. F. (green) is the nonpurified commercial product with a purity of about 98 per cent. The purified drug, phenothiazine N. F. (purified), is a pale yellow product. An impurity found in most commercial forms of the green drug is organically bound iodine, which is incorporated during the use of this element as a catalyst in the manufacturing process (H. I. Nachimson *et al.*, *Proc. Soc. Exp. Biol. Med.* **87,** *157 (1954)*).

This observation led to a comparison of the depression of radioactive iodine uptake by thyroid in rats receiving equimolar amounts of iodide, either as potassium iodide or as phenothiazine N. F. (green). The latter contained 0.4 per cent of iodine. Except for minor differences, the two sources of iodine resulted in equal depression of thyroid uptake of a test dose of radio-iodine. Since the radio-iodine was administered simultaneously with the test materials and the radioactivity of the thyroid was determined five hours later, there was little doubt that, if the activity of the drug was due to iodide, the iodine must have been liberated rapidly from its organically bound form.

Partial separation of the commercial drug with column chromatography and assay of the fractions again indicated that the fraction containing the iodine inhibited radio-iodine uptake by the thyroid. The inhibition was again equal to that obtained with equimolar amounts of inorganic iodide.

Further studies included determination of radioactive iodine uptake by the thyroid following test doses of purified phenothiazine, phenothiazine sulfoxide, diphenylamine, and phenothiazine N. F. (green), bromine catalyzed. With the exception of purified phenothiazine, none of these compounds had any effect. The purified phenothiazine, however, suppressed radio-iodine uptake far out of proportion to the amount expected from the iodine present.

Throughout these studies, when sufficient inorganic iodide was fed to produce a suppression of radio-iodine uptake by the thyroid, elevated iodide excretion in the urine was observed. In contrast, equimolar levels of iodide in the form of phenothiazine, which produced an equivalent suppression of radio-iodine uptake, did not result in any increase in urinary iodide. It thus appeared that commercial phenothiazine N. F. (green) contains, as an impurity, an unknown factor or factors capable of suppressing radio-iodine uptake. The iodine known to be present in the unpurified drug may have produced the observed results by iodine saturation of the thyroid gland or by dilution of the radio-isotope, but the possibility of a true thyroid depressant was not excluded.

A further study of this problem involved the use of a number of commercial preparations of the drug, both purified and green, and the results lend support to the view that at least two factors are operative in depressing thyroidal radio-iodine uptake (R. H. Wasserman *et al.*, *Am. J. Vet. Res.* **17,** *149 (1956)*). These observations confirmed those of Nachimson and associates in that the major inhibition of thyroidal radio-iodine uptake could be accounted for on the basis of iodide present in the unpurified drug. These workers further established that phenothiazine synthesized with I^{131} as catalyst resulted in the thyroid uptake of labeled iodine equal to that observed with labeled potassium iodide.

Purified phenothiazine was again observed to inhibit thyroidal iodine uptake, although it was far less potent than inorganic iodide. These two reports are thus in agreement, and merit the tentative conclusion that iodide ion alone cannot account for all of the thyroid-depressant effects of phenothiazine (green).

These studies merit the attention of nutrition investigators who have used phenothiazine N. F. (green) as an ingredient in the rations of livestock for the control of parasites. Whether the thyroidal iodine-uptake effects of this drug are of sufficient magnitude to influence tissue metabolism is not clear. In the studies of Nachimson and associates, levels of the drug that effected a 50 per cent reduction in radio-iodine uptake by the thyroid failed to influence the weight of the gland.

DISEASE RESISTANCE AND HIGH VITAMIN INTAKES

Little attention has been given to the possibility that high levels of certain vitamins in the diet may actually decrease the ability of the host to withstand invasion by infectious organisms. Investigations on the role of vitamins in resistance to disease have been chiefly concerned with the deficiency state, which is poorly correlated with resistance to disease, being positive in some cases and negative in others (J. G. Wooley and W. H. Sebrell, *Pub. Health Reports* **57,** *149* (*1942*); *Nutrition Reviews* **1,** *66* (*1943*); **2,** *327* (*1944*); **3,** *179*, *229* (*1945*); **4,** *21* (*1946*); **9,** *245* (*1951*); L. Reiner and J. B. Paton, *Proc. Soc. Exp. Biol. Med.* **30,** *345* (*1932*); *Nutrition Reviews* **2,** *261* (*1944*); **3,** *69* (*1945*)).

In a different approach to this problem, C. H. Hill and H. W. Garren (*Ann. N. Y. Acad. Sci.* **63,** *186* (*1955*)) have tested the effect of high dietary levels of all the known vitamins on resistance of newly hatched chicks to fowl typhoid. The experimental period was divided into two parts—a preinoculation period of four weeks and a postinoculation period of approximately three weeks. At 4 weeks of age, the birds were inoculated (orally) with a culture of *Salmonella gallinarum*. The resulting disease was allowed to run its natural course, and the experiments were terminated when the chicks had ceased to die and appeared to be recovering rapidly from the effects of the infection.

The first experiment showed that inclusion of high dietary levels of all the known vitamins (ten times minimum daily requirement) increased the percentage of surviving animals from 10 to 40 and increased the average survival time. Studies were next undertaken to determine the effect of groups of vitamins on resistance to fowl typhoid. The vitamins were arbitrarily divided into the fat-soluble group, the water-soluble B-vitamin group, and ascorbic acid. These groups showed no beneficial effects by themselves; in fact, high levels of the fat-soluble group (A, D, E and K) considerably decreased both total survival (from 48 per cent to 15 per cent) and average survival time (from 12.2 days to 8.6 days). These results indicate that the effect of high levels of vitamins on resistance to fowl typhoid is due to some combination of vitamins distributed among the groups tested and further suggest that some of the vitamins are acting contrary to the defenses of the host.

Investigations directed toward determining which of the vitamins were necessary, unnecessary, or harmful were carried out using the high-vitamin supplement of all vitamins except the one under test, which was present at the same level as in the basal diet.

All the fat-soluble vitamins, vitamin B_{12}, pyridoxine and ascorbic acid were found to be required in the vitamin mixture, but the absence of high levels of thiamine, biotin, folacin, or pantothenic acid apparently increased the resistance of the chicks. To test the possibility that the high level of vitamin supplement used throughout these studies may have been too high for maximum resistance, chicks were given two

and one-half, five, seven and one-half, and ten times the level of all vitamins present in the basal diet. It was found that a vitamin supplement which was five times greater than that of the basal diet resulted in maximal survival and greatest average survival times.

Since it is difficult to interpret these results in terms of decreased resistance of the host, it seemed probable that excessively high total vitamin supplements, as well as the oil-soluble vitamins as a single supplementary group, and thiamine, biotin, folacin, and pantothenic acid alone, had two effects on the disease system: one on the host, resulting perhaps in an increased resistance to infectious processes, and the other on the *Salmonella* organism, increasing its ability to survive and grow. To evaluate this hypothesis, various levels of the vitamin supplement were fed, and the chicks were infected both orally (as in all previous experiments) and intramuscularly. Unfortunately, the injected animals had a greater mortality rate than did orally inoculated birds, and, in addition, the highest level of vitamin supplement (ten times that of the basal diet) resulted in the greatest number of survivors. Further studies must be made, with still higher levels of vitamins, to determine whether a plateau in response to vitamins can be reached when the host is inoculated intramuscularly. The authors feel that if such a plateau is reached, and if further increases in vitamin levels of the diet do not result in a lowering of the survival rate, this will be evidence that the apparent decrease in resistance occurring when the highest vitamin levels are fed to birds inoculated orally is due to effects of high levels of certain vitamins on the infectious agent and not on the host.

OBESITY FROM HIGH FAT DIETS

The assumption that animals do not develop obesity under normal conditions of liberal feeding has been challenged in recently published results by O. Mickelsen, S. Takahashi, and C. Craig (*J. Nutrition* **57,** *541 (1955)*). These workers compared the growth rate and obesity in rats of three strains under high fat, low fat and average dietary conditions. The high fat diet consisted of the following (in per cent): casein, 25; sucrose, 7; starch, 1; salts, 4; hydrogenated vegetable oil, 60; and cottonseed oil, 3. The starch served as the B-vitamin carrier and was supplemented with the following vitamins (in milligrams per gram): thiamine hydrochloride, 0.2; riboflavin, 0.3; pyridoxine hydrochloride, 0.25; calcium pantothenate, 2.0; niacin, 2.0; choline chloride, 100; inositol, 10; biotin, 1.0; and folacin, 0.1. The cottonseed oil was fortified to supply 5.5 and 1.1 I.U. of vitamins A and D, respectively, and 0.5 mg. of alpha-tocopherol acetate per gram of diet.

Experimental changes in the high fat diet included doubling the B-vitamins, inclusion of whole liver powder (5 per cent level), addition of penicillin (40 mg. per cent), and addition of vitamin B_{12} (2 micrograms per 100 g.). None of these changes influenced the results.

The low fat diet was the same as the high fat diet described above, except that the casein was present at the 30 per cent level and the hydrogenated vegetable oil was replaced with an equal weight of sucrose. A diet designated "Best Low-Fat," developed by the laboratory, was also included in the study. This was essentially the low fat diet with additional supplements of cystine (0.3 per cent), aureomycin (0.1 per cent), niacin (2 mg. per cent) and vitamin B_{12} (20 micrograms per cent).

In an initial experiment weanling rats of the Osborne and Mendel strain attained body weights of 429, 492 and 726 g. on a stock diet, the low fat diet and the high fat

diet, respectively, in twenty-five weeks (5 rats per treatment). At forty-one weeks the 3 surviving rats on the high fat diet averaged 968 g., compared with average weights of 504 and 579 g. in animals on the remaining two treatments.

Some strain differences were noted in response to the high fat diets. For example, animals of the Osborne-Mendel strain averaged 666 g. in weight whereas rats of the Sprague-Dawley strain averaged only 464 g. twenty-five weeks after weaning. The Sprague-Dawley strain appeared to be highly susceptible to middle ear or upper respiratory infections under high fat feeding. Animals of the National Institutes of Health-Black strain also appeared to gain weight more slowly than the Osborne-Mendel strain under high fat dietary conditions.

Even though the above strain differences were noted, animals of all strains exhibited a tendency toward frank obesity under high fat feeding. Summarizing the data for all 60 rats fed the high fat diets, the authors noted the following: 21 animals (35 per cent of the starting group) attained weights of 1000 g. or over. An equal number attained weights of 800 and 1000 g. Maximum weight attained in any animal was 1655 g., compared to 555 g. for its control on the stock diet. It was thus evident that under these conditions of high fat regimen with liberal accessory-factor supplementation, about 70 per cent of the animals became obese.

The maximum obese condition generally appeared between the fortieth and fiftieth week after weaning. Following a plateau in body weight of varying duration, all obese rats finally lost weight at the rate of 30 to 50 g. weekly. Death usually occurred shortly after the weight loss began.

Obesity has been produced in a variety of ways. Hypothalamic lesions bring about a dramatic pathologic increase in food intake and obesity in a number of species, including the rat (*Nutrition Reviews* **2,** *105* (*1944*); **9,** *157* (*1951*); **10,** *280* (*1952*); **11,** *55* (*1953*)). Goldthioglucose injection into mice produces obesity in this species (*Ibid.* **13,** *15* (*1955*)), and a hereditary obesity occurs in C_3H-strain mice fed high fat diets (*Ibid.* **10,** *303* (*1952*); **11,** *88* (*1953*)). Forced feeding or thyroidectomy will likewise result in extra fat deposition. With all of these procedures, however, an interpretation of obesity must be conditioned by complicating influences of the experimental technic.

Mention should be made of the observation of D. J. Ingle (*Proc. Soc. Exp. Biol. Med.* **72,** *604* (*1949*)) that rats raised in small cages attained weights as high as 1000 g. when fed a semiliquid diet and no water other than that in the diet. This worker employed a diet of casein, starch, dextrin, sucrose, yeast, salts, vitamins and water.

A smaller cage that would limit exercise did not appear to hasten or increase obesity in the studies carried out by Mickelsen and his associates. An interesting further observation was the failure of any evidence of ketosis to appear on the high fat diets (85 per cent calories from fat) in animals that became extremely obese.

Mickelsen and his associates are of the opinion that genetic or hormonal disturbances were not responsible for the obesity produced under their conditions. A craving for a missing dietary factor with resultant increase in food consumption seemed to be precluded as a reasonable explanation for the development of obesity, since addition of whole liver powder or other vitamins failed to influence the results.

These results support the view that a high percentage of rats can become obese simply by consuming excessive calories under dietary conditions that appear to satisfy all nutritional requirements and in which 85 per cent of the calories are supplied in the form of fats.

NOTES

Letter to the Editor

Dear Sir:

We have read with interest the review on calcium intake and rickets (*Nutrition Reviews* **14,** *36* (*1956*)). While it is always questionable to apply data obtained with one species to the nutritional problems of another species, there are pertinent data on calcium intake of chicks and turkey poults. J. S. Nowotarski and H. R. Bird (*Poultry Sci.* **22,** *72* (*1943*)) showed that increasing dietary calcium and phosphorus decreased the vitamin D requirement. A calcium: phosphorus ratio of 2:1 gave better calcification than did a ratio of 1.1:1. Increasing the calcium content of the A.O.A.C. chick vitamin D assay diet improved response to given levels of vitamin D (J. C. Fritz and H. R. Halloran, *Ibid.* **22,** *314* (*1943*)).

In work with turkey poults (J. C. Fritz, J. H. Hooper, and H. P. Moore, *Poultry Sci.* **24,** *324* (*1945*)), the level of dietary calcium was shown to have a marked influence on calcification. Rickets was prevented, under the experimental conditions, either by increased calcium or by increased vitamin D_3.

The rat experiments of H. Steenbock and D. C. Herting (*J. Nutrition* **57,** *449* (*1955*)) showed that both growth and calcification were influenced by the calcium intake and the calcium:phosphorus ratio. Within limits, the results of an unfavorable calcium:phosphorus ratio could be counteracted by vitamin D.

The calcium:phosphorus ratio of dry, fat-free bone is near 2:1. Since the chief need for calcium in the growing young is for bone formation, we find it hard to accept recommendations for low calcium diets, and especially those in which the calcium:phosphorus ratio is less than unity.

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Editorial Comment:

On the basis of the studies in other species it is undoubtedly true that one would expect to find more rickets when the calcium intake is low. The point emphasized by the review, however, is that the clinical evidence does not support this expectation. The efficiency of calcium and phosphorus utilization would probably be better in man, as it is in animals, if the ratios in the diet approximated the need for tissue formation. Such evidence is not available for human beings.

Creatine in Progressive Muscular Dystrophy

The disease progressive muscular dystrophy is accompanied by a marked creatinuria. It has been thought that this creatine comes from muscle (which is undergoing atrophy), but experiments conducted by J. D. Benedict *et al.* (*J. Clin. Invest.* **34,** *141* (*1955*)) indicate that this may not be the case. Three patients with muscular dystrophy were studied (one previously reported on by Benedict *et al.*, *Metabolism* **1,** *3* (*1952*)) who were given glycine labeled with heavy nitrogen (N^{15}) as a precursor of creatine. If, as had been believed, creatine and creatinine in the urine both come from muscle creatine, they should have had the same "isotope enrichment" after feeding of N^{15}-glycine. This was not the case in these 3 patients, the urinary creatine having a much higher concentration of N^{15} than that of creatinine, at least initially. The creatine N^{15} excess fell gradually so that the two (creatine and creatinine) were essentially the same by the eighth or tenth day after glycine administration. The authors believe that these data support the hypothesis that urinary creatine in progressive muscular dystrophy does not come from muscles but is "freshly synthesized." They did, however, find a significant concentration of urinary creatinine labeled with N^{15} suggesting, as they say, that muscle in this disease can still convert creatine into creatinine in normal or even increased quantities.

Another interesting hypothesis these authors make from their studies is that there may be two reservoirs of glycine in the body (presumably in the liver), one of which is available for the synthesis of creatine and the other for the synthesis of hippurate from benzoic acid. This conclusion was derived from data obtained after the administration of N^{15}-glycine and small quantities of benzoic acid to these patients. It was found that a higher isotope concentration of N^{15} appeared in the creatine than in the hippurate. This is an interesting conclusion and, as the authors point out, if there are two nonhomogeneous depots of glycine in the liver, it makes for some difficulty in interpreting data from isotope experiments in which the assumption of homogeneous distribution must be made.

Influence of Sugars on Utilization of Protein Hydrolysate

Glucose and similar sugars form combinations with amino acids and it has been suggested that these compounds may not be available for metabolism, rendering the amino acid solution or protein hydrolysate less useful nutritionally. A careful study of this phenomenon has been made by H. N. Christensen, P. B. Wilber, B. A. Coyne, and J. H. Fisher (*J. Clin. Invest.* **34,** *86 (1955)*).

Three protein hydrolysates were studied, one of fibrin, a second made from casein and pork pancreas, and a third from plasma proteins. The extent of aminoaciduria and peptiduria after intravenous infusion compared with the amino acid and peptide content of the infusion was studied in a group of patients convalescing from surgical operations and in normal individuals.

The loss of peptides in the urine was found to be increased when the protein hydrolysate was autoclaved with glucose as compared to the effect on the same hydrolysate when glucose was added after autoclaving or when no sugar was present. The presence of sugar itself did increase the excretion of peptides and of amino acids slightly, though not to the extent that the peptides were lost when the hydrolysate had been autoclaved with glucose. Autoclaving with glucose had a greater effect on peptide excretion than when fructose was used. Approximately 5 per cent of the infused free amino acids and nearly 30 per cent of the peptides were lost when the hydrolysate was autoclaved with glucose. Such a loss would certainly be nutritionally important.

The authors recommend that instead of autoclaving sugars with the hydrolysates in their commercial preparation the two solutions might better be mixed under sterile conditions immediately before infusion.

Recent Nutrition Books

Family Diet and Health in Pre-War Britain. A Dietary and Clinical Survey. Report to the Carnegie United Kingdom Trust from the Rowett Research Institute. Carnegie United Kingdom Trust, Comely Park House, Dunfermline, Fife, Scotland. Pp. 164.

Studies on Expenditure of Energy and Consumption of Food by Miners and Clerks, Fife, Scotland, 1952. R. C. Garry, R. Passmore, Grace M. Warnock, and J. V. G. A. Durnin. Medical Research Council, Special Report Series No. 289, Her Majesty's Stationery Office, York House, Kingsway, London, W.C. 2, 1955. Pp. 70. Price: 5 shillings net.

Joint FAO/WHO Expert Committee on Nutrition, Fourth Report. World Health Organization, Technical Report Series No. 97, 1955. Columbia University Press, New York, N. Y. Pp. 58. Price: $.60.

International Survey of Programmes of Social Development. Bureau of Social Affairs Report. Prepared in cooperation with FAO, WHO, ILO, and UNESCO, Published by Columbia University Press. 2960 Broadway, New York 27, New York. Pp. 219. Price: $2.00.

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